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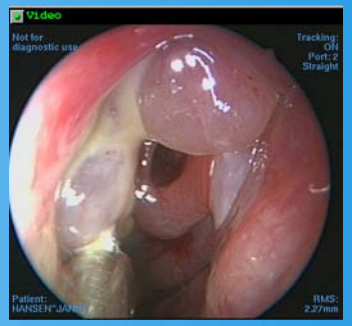
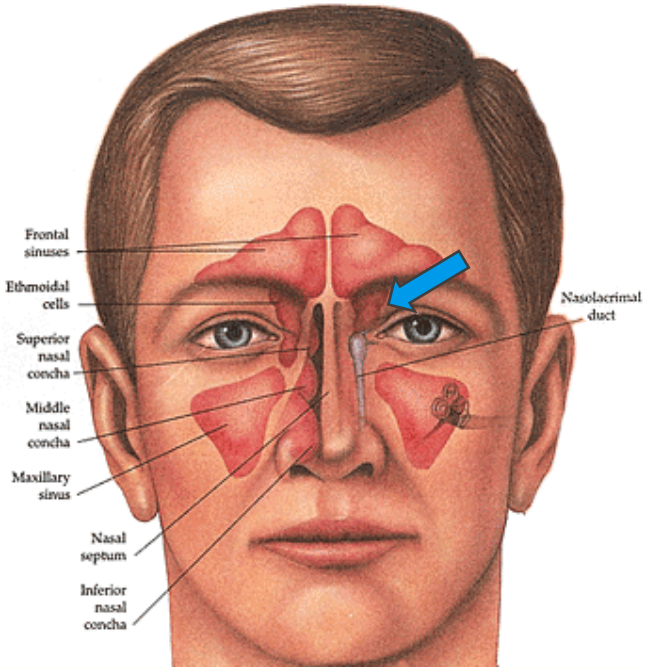
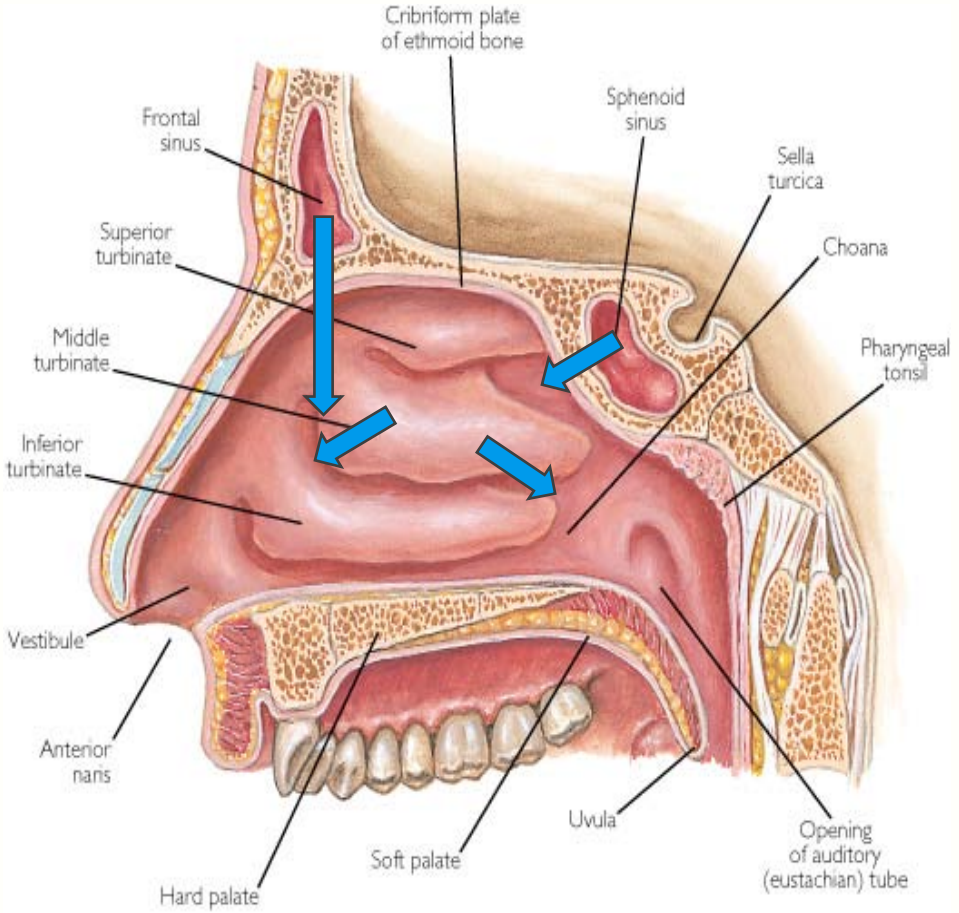
New insights into the microbiology of the CF lung: “United airways?”



Helle Krogh Johansen, MD, DrMedSci
Department of Clinical Microbiology
Rigshospitalet, Denmark
hkj@cochrane.dk

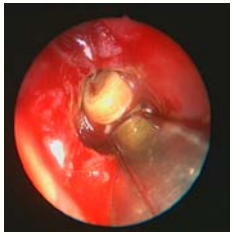
Sinus anatomy

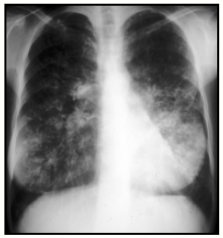




Early colonisation: sinuses versus lungs

- Different types of intermittent colonisation
  - Every colonisation is with a new environmental *P. aeruginosa* strain
  - Multiple recurrent colonisations are with the same genotype. Low number of bacteria in sputum. The sinuses probably constitute a persistent focus that may provide extended opportunities for localised evolution
- Antibiotic therapy efficiently removes *P. aeruginosa* from the lungs
  - The direction of the migration is mainly downwards at the early stages
- Obstructed sinus cavities lead to
  - Changes in nutrient utilization, antibiotic resistance, reduction in growth rate



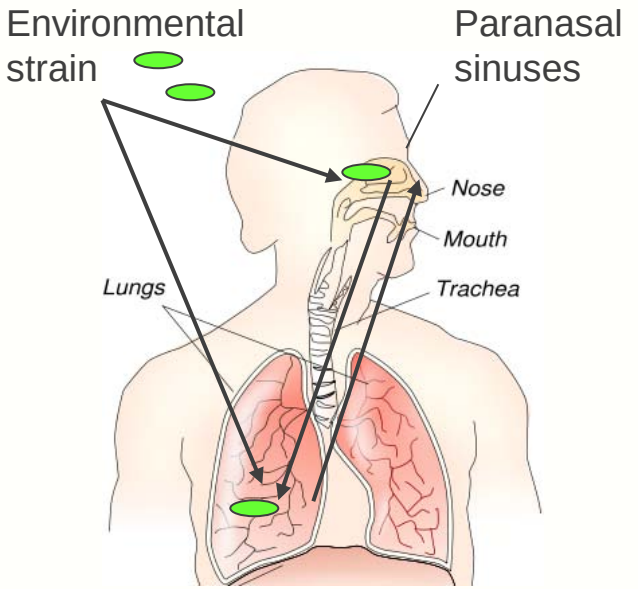


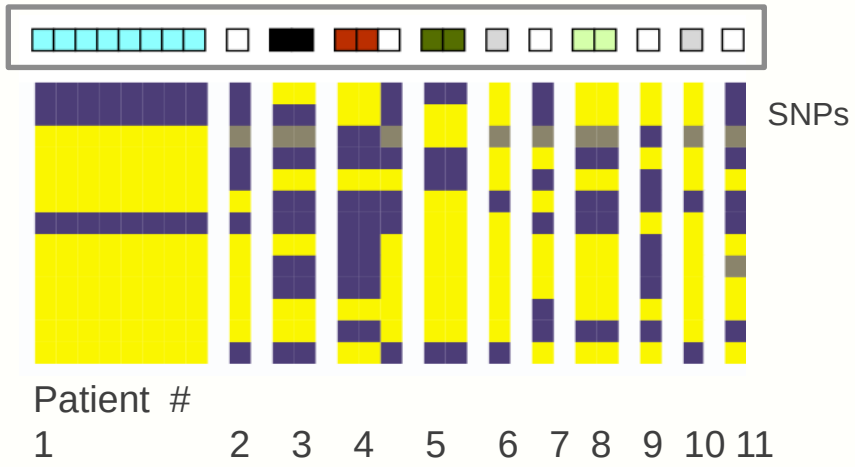
Environment | Sinuses | Lungs

Unique genotypes, fast growth, non-mucoid, antibiotic-susceptible

Antibiotic resistance, mucoid, loss of QS and motility, biofilm formation

Adaptation / evolution





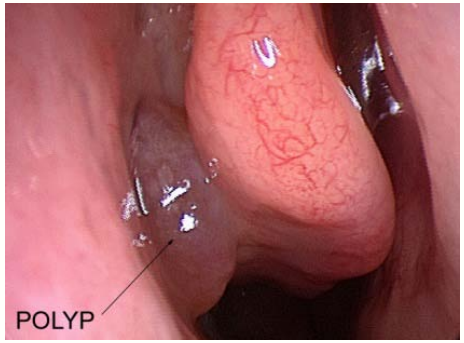
Jelsbak L et al. I&I 2007

What do we know about the sinuses in CF?

- The paranasal sinuses are virtually non-vented, hollow organs with poor perfusion, that are protected by the filtration function provided by the nose

- Most CF patients have chronic rhinosinusitis (>12 w) - but it has been neglected for decades when compared to the lung symptoms

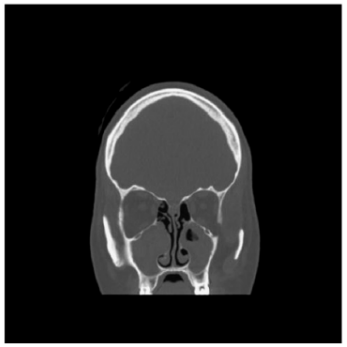
- Symptoms:
  - Nasal obstruction and polyps
  - Facial pressure
  - Rhinorrhoea
  - Loss of smell
  - Tiredness and fatigue



- Mechanisms that contribute to chronic rhinosinusitis: mucus stasis and impaired mucociliary transport (basic CFTR defect)

What do we know about the sinuses in CF?

- "United airways": sinuses and lungs have similar physical properties
- Mechanical obstruction of the sinus ostia by thickened static mucus and chronic inflammation ⟹ decreased O_2 content and reduced CFTR expression leading to less functional immune system
- Anaerobic environment ⟹ upregulation of alginate production and biofilm formation, QS-deficient mutants
- Inflammation differs in the sinuses and lungs
- Partially obstructed sinus cavities lead to reduced access of administered antibiotics and increased antibiotic resistance

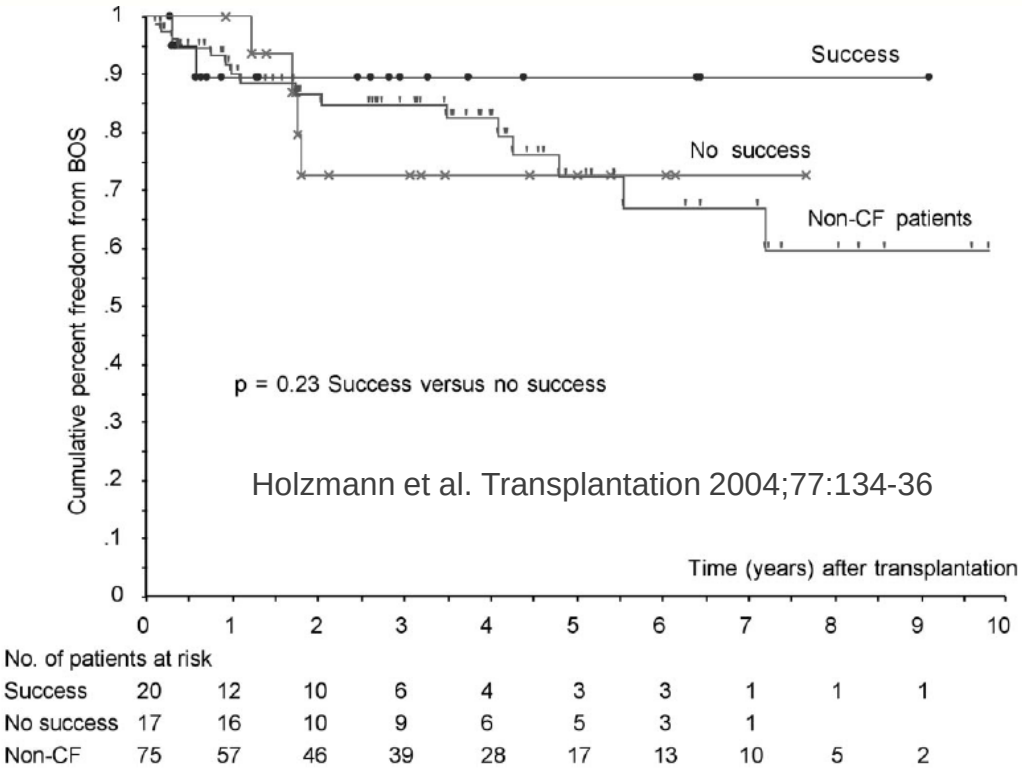


- Stationary bacterial population are prone to adaptation and evolution

Table 2 Comparison of posttransplant BAL and sinus aspirate cultures among CF lung transplant recipients

Bacteria	BAL (n = 68 patients)	Sinus aspirate (n = 44 patients)	
Pseudomonas sp.	87%	82%	$p = 0.59$
Staphylococcus sp.	40	27	$p = 0.22$
Streptococcus sp.	24	14	$p = 0.23$
Stenotrophomonas sp.	19	9	$p = 0.18$
Burkholderia sp.	4	5	$p = 1.00$

Leung et al. Am J Rhinology 2008;22,192-96



Holzmann et al. Transplantation 2004;77:134-36

FIGURE 1. Kaplan-Meier estimates of the cumulative incidence of freedom from bronchiolitis obliterans syndrome (BOS) in relation to the result of sinus management and compared to patients without cystic fibrosis (non-CF).

Lung tx patients

Table 2 Molecular epidemiology of Pseudomonas aeruginosa in lung allografts of lung transplant recipients with cystic fibrosis

	*P aeruginosa genotypes**		
Patient no.	*Before transplantation*[1]	*After transplantation*[2]	*Six months after transplantation*[2]
1	ab, ac, ad	ab, ac	ab
2	C, af, ag	C	C
3	ah	ah	ah
4	ai	ai	ai
5	aj	aj	aj
6	ak	ak	ak
7	al, am	al, am	al
8	an	an	an
9	ao, ap	ao	ao
10	aq	aq	aq
11	ar	ar	ar

[1] Sputum or throat swab; [2] Bronchoalveolar lavage fluid.
* Clones and clonal variants already identified in recent studies[10] are indicated with the same capital letter. Novel genotypes were given two small letters.

Walter et al 1997;52:318-21

Prospective study, 2009

Simultaneously nasal lavage and sputum culture (N=187)

86% (31/36) of *S. aureus* and 95% of *P. aeruginosa* strain pairs were genotypically identical (*spa* or SNPs)

The UAW play a role as a reservoir of *S. aureus* and *P. aeruginosa*

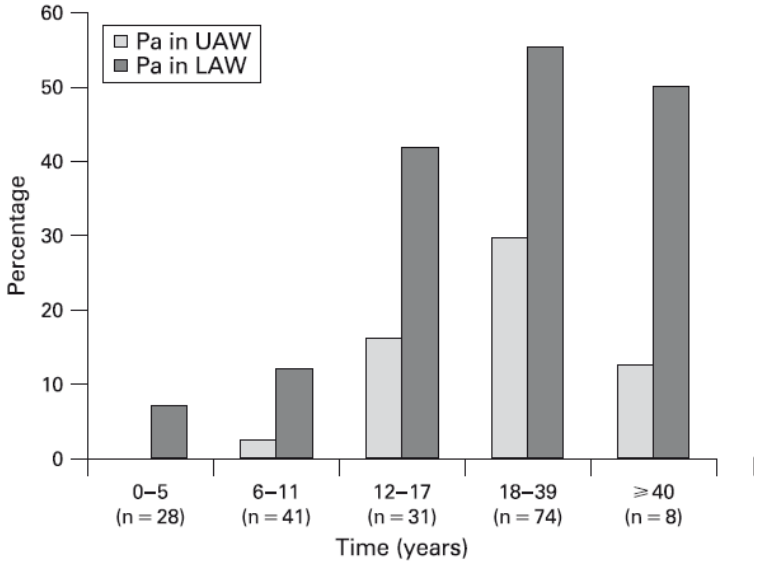
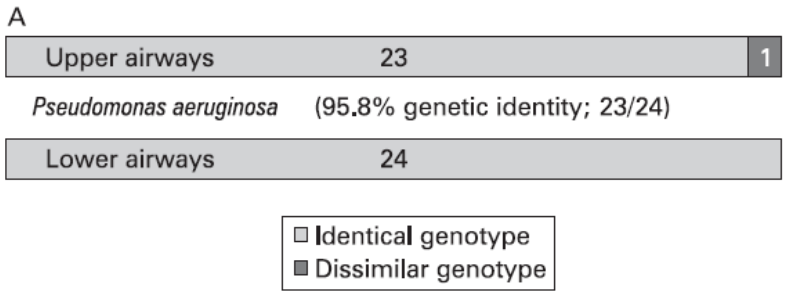


Figure 2 Fraction of patients with detection of *Pseudomonas aeruginosa* (Pa) in the upper (UAW) and lower airways (LAW) in relation to their age (n = 182).



Mainz JG et al. Thorax 2009;64:535-40

Prospective study 2010

Simultaneously UAW and LAW culture (N=157)

ELSEVIER

Journal of Cystic Fibrosis 9 (2010) 130 – 134

Journal of Cystic Fibrosis
www.elsevier.com/locate/jcf

Short Communication

Upper and lower airway cultures in children with cystic fibrosis: Do not neglect the upper airways

Hilde J.C. Bonestroo, Karin M. de Winter-de Groot, Cornelis K. van der Ent, Hubertus G.M. Arets *

Department of Paediatric Respiratory Disease, Cystic Fibrosis Centre Utrecht, Wilhelmina Children's Hospital, KH 01.419.0, University Medical Centre Utrecht, P.O. Box 85090, 3508 AB Utrecht, The Netherlands

Received 5 October 2009; received in revised form 23 December 2009; accepted 4 January 2010
Available online 27 January 2010

Distribution of bacteria in lower and upper airway cultures (n=157).

Microorganisms	LAW cultures	UAW cultures	p-value
Total positive cultures	125 (79.6%)	69 (43.9%)	<0.001
P. aeruginosa	54 (34.4%)	18 (11.5%)	<0.001
S. aureus	79 (50.3%)	42 (26.8%)	<0.001
H. influenzae	17 (10.8%)	17 (10.8%)	1.00
E. coli	4 (2.5%)	2 (1.3%)	0.63
S. maltophilia	5 (3.2%)	0 (0%)	0.06
K. oxytoca	3 (1.9%)	2 (1.3%)	1.00
K. pneumoniae	2 (1.3%)	0 (0%)	0.50
E. cloacae	3 (1.9%)	1 (0.6%)	0.63
B. cepacia	1 (0.6%)	0 (0%)	1.00
S. pneumoniae	0 (0%)	3 (1.9%)	0.25

Data are given as number of positive cultures and percentage of total cultures.
N: number of patients. LAW: lower airways. UAW: upper airways.

”*P. aeruginosa* positive UAW cultures appeared to precede positive LAW cultures in a substantial part of patients suggesting some kind of cross-infection between UAW and LAW”

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Journal of Cystic Fibrosis xx (2012) xxx – xxx

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Original Article

Colonisation and infection of the paranasal sinuses in cystic fibrosis patients is accompanied by a reduced PMN response ☆

Helle Krogh Johansen [a,*], Kasper Aanaes [b], Tania Pressler [c], Kim Gjerrum Nielsen [c], Jacob Fisker [b], Marianne Skov [c], Niels Høiby [a,d], Christian von Buchwald [c]

[a] *Department of Clinical Microbiology, afsnit 9301, Rigshospitalet, Juliane Maries Vej 22, DK-2100 Copenhagen Ø, Denmark*
[b] *Department of Otolaryngology, Head and Neck Surgery, afsnit 2072, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark*
[c] *CF Centre Copenhagen and Paediatric Pulmonary Service, Department of Paediatrics and Adolescent Medicine, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark*
[d] *Institute of International Health, Immunology and Microbiology, Panum Institute, University of Copenhagen, Blegdamsvej 3B, 2200 Copenhagen N, Denmark*

Received 11 February 2012; received in revised form 12 April 2012; accepted 26 April 2012

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78 CF patients included (FESS)

21 chronically infected with PA
18 growth of PA in sinuses and lungs, 100% genetically identical

31 intermittently colonised wirh PA
21 growth of PA in sinuses and lungs, 91% genetically identical

Johansen HK et al. JCF 2012

Copenhagen “principles” since 2009 for CF endoscopic sinus surgery

- Intermittently colonized patients with **declining lung function** and **increasing antibodies**. Patients with an **unknown focus** and increasing antibodies against *Pseudomonas aeruginosa, Achromobacter xylosoxidans* or *Burkholderia cepacia complex* are given priority

- Patients who have had **lung transplantation** are offered surgery within the first year following the transplantation

- Patients with severe **symptoms** of Chronic Rhinosinusitis in accordance with the EPOS (European Position Paper On Rhinosinusitis And Nasal Polyps 2012) guidelines.

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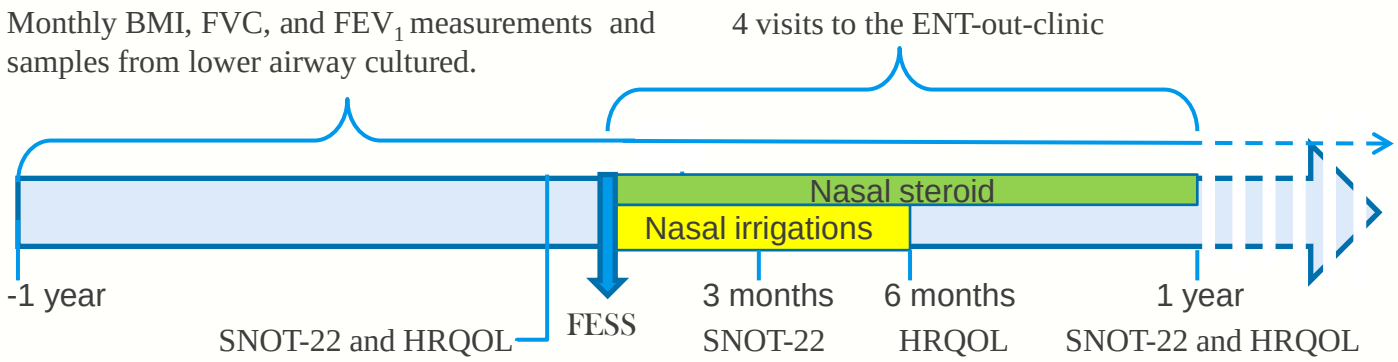
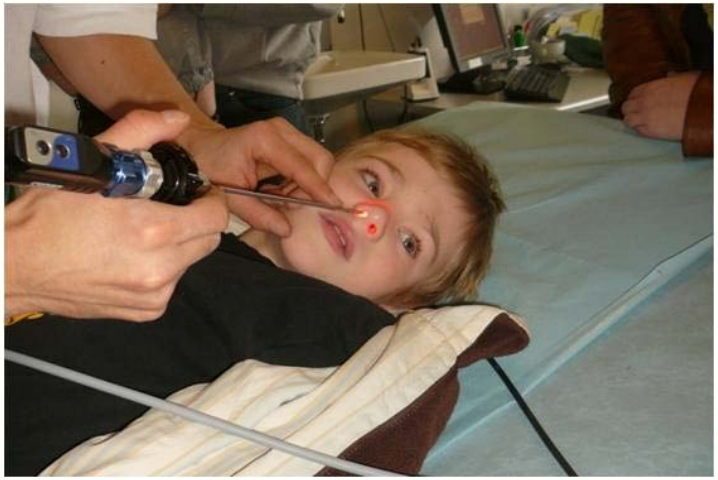
Follow-up and post surgery protocol

- *Treatment* after sinus surgery





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Otolaryngol Head Neck Surg. 2009 September ; 141(3): 358–363. doi:10.1016/j.otohns.2009.05.034.

Outcomes of Sinus Surgery in Adults with Cystic Fibrosis

Ayesha N. Khalid, MD, **Jess Mace, MPH**, and **Timothy L Smith, MD, MPH**
Division of Rhinology and Sinus Surgery, Oregon Sinus Center, Department of Otolaryngology – Head and Neck Surgery, Oregon Health and Science University, Portland, Oregon

Conclusions: While baseline measures of disease-severity are worse in the CF population, our data support objective and QoL improvements for adult patients with co-morbid CF comparable to patients without CF.

Pro-Cons

ORIGINAL ARTICLE

Cystic Fibrosis and Endoscopic Sinus Surgery

Relationship Between Nasal Polyposis and Likelihood of Revision Endoscopic Sinus Surgery in Patients With Cystic Fibrosis Arch Otolaryngol Head Neck Surg 2010

Scott Rickert, MD; Victoria E. Banuchi, MD; Joan D. Germana, MD; Michael G. Stewart, MD, MPH; Max M. April, MD

Table. Modified Malm Polyp Scale Comparing Revision ESS Among Grades, Total Revision ESS Procedures, and Time Between Procedures

Polyp Grade[a]	Patients, No. All (n=49)	Patients, No. Revision ESS (n=14)	Total Revision ESS Procedures, No.	Time Between Procedures, Mean (SD), mo
A	16	0	0	NA
B	14	3	3	39.7 (22.3)
C	19	11	25	23.8 (19.4)

ESS does not result in prompt disease recurrence and need for revision surgery. The overall rate seem lower than might have been predicted

Reduced O_2 in CF sinuses

Catheter O_2 optode for measuring oxygen tension

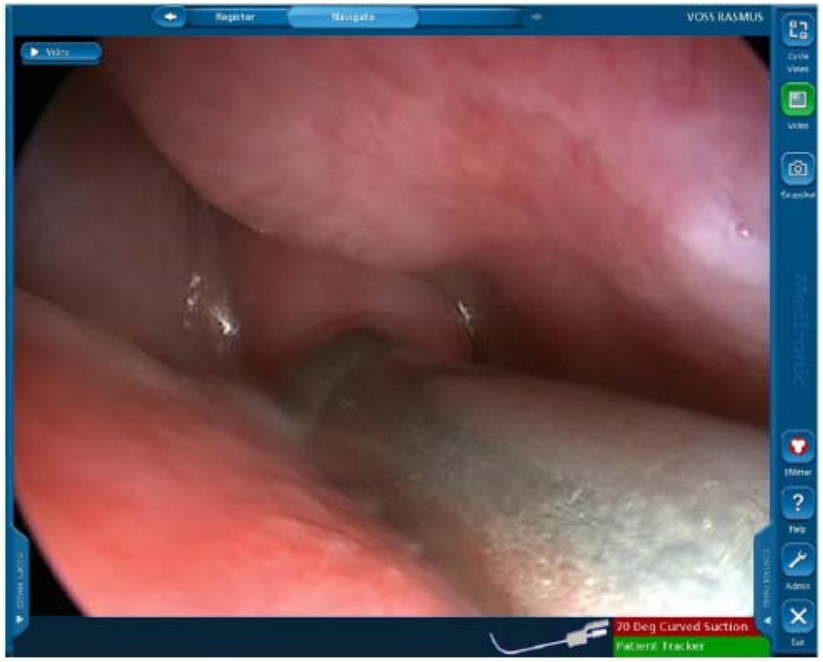
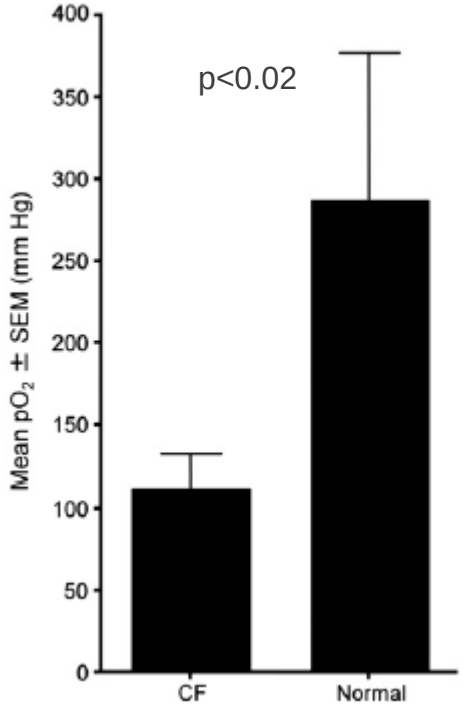


Fig. 2. The catheter on its way to the right maxillary sinus.

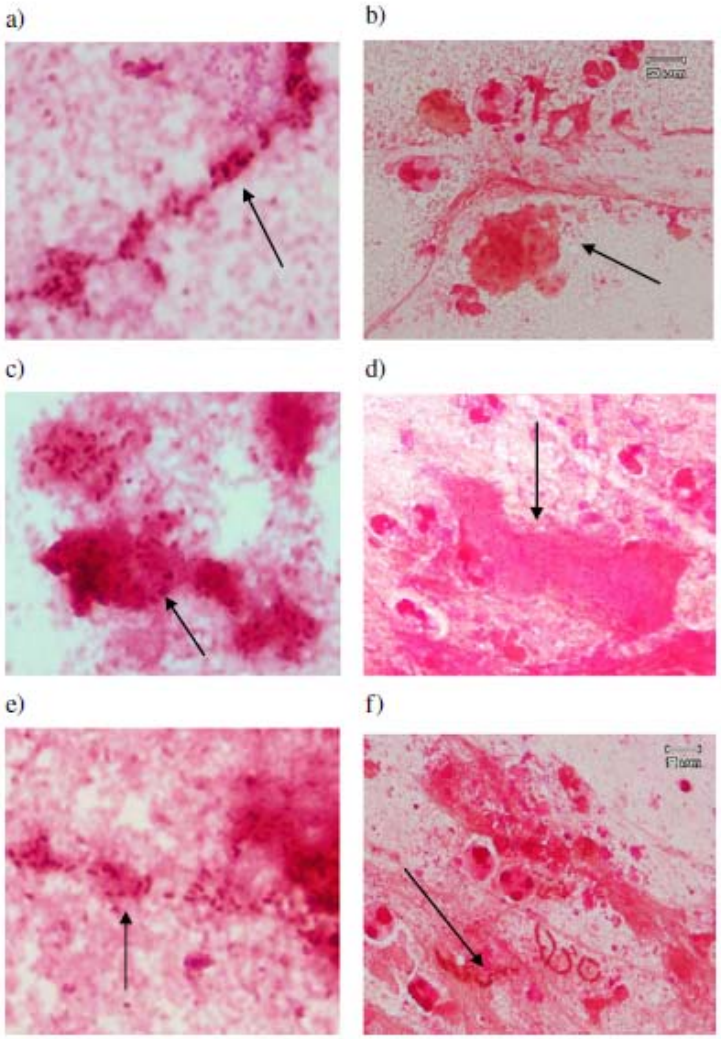
- *P. aeruginosa* can adapt to the microaerophilic/anaerobic environment in the sinuses
- Upregulation of alginate production
- Less well functioning immune system in O_2 depleted areas



Aanaes K et al.: JCF 2011;10:114-20

P. aeruginosa biofilms: different location - different immune response

Biofilms in <u>sinuses</u>
No PMNs

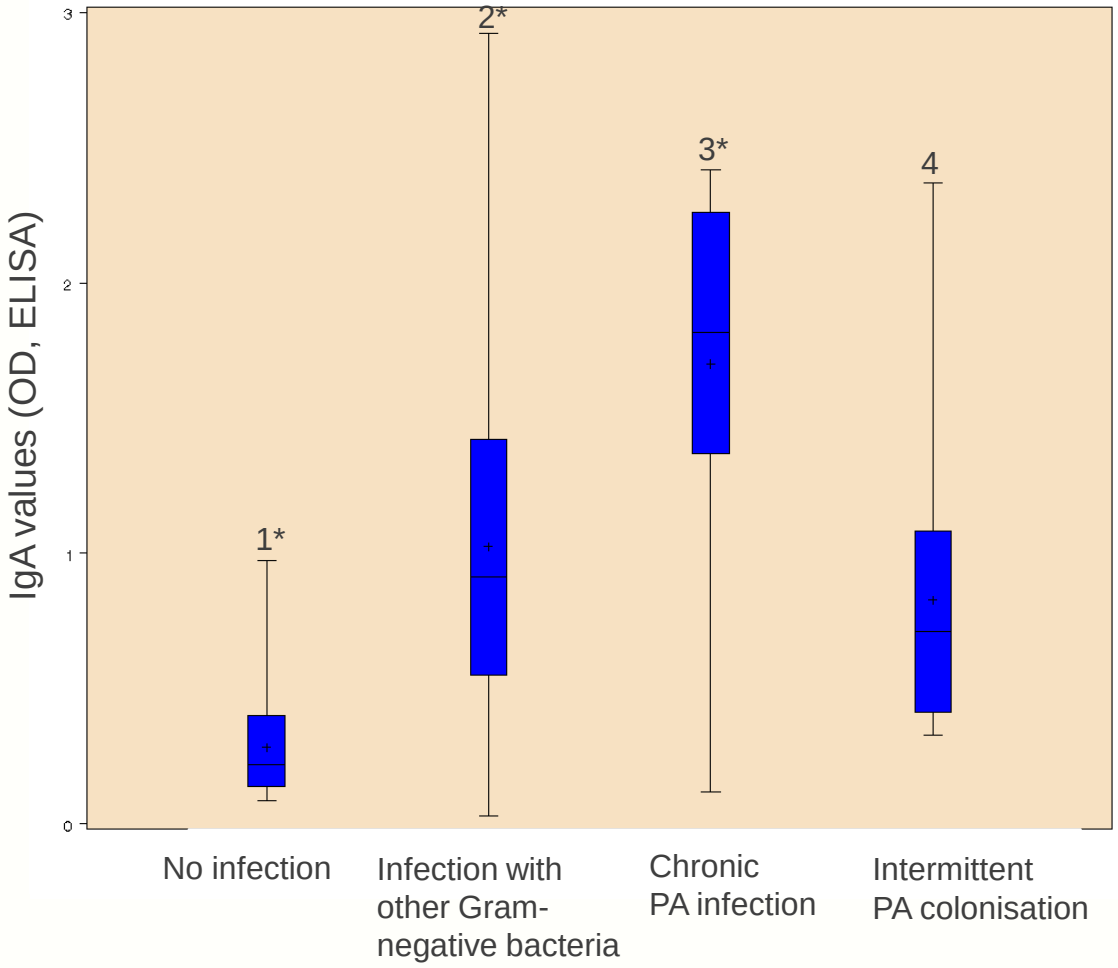


Biofilms in the <u>lungs</u>
Many PMNs

Johansen HK et al. JCF 2012

Fig. 1. a–f. Microscopic investigation of Gram-stained smears of pus from the sinuses (a, c and e) and corresponding sputum (b, d and f) obtained from three patients chronically infected with *P. aeruginosa* at the time of sinus surgery, magnification ×1000. Arrows indicate biofilms.

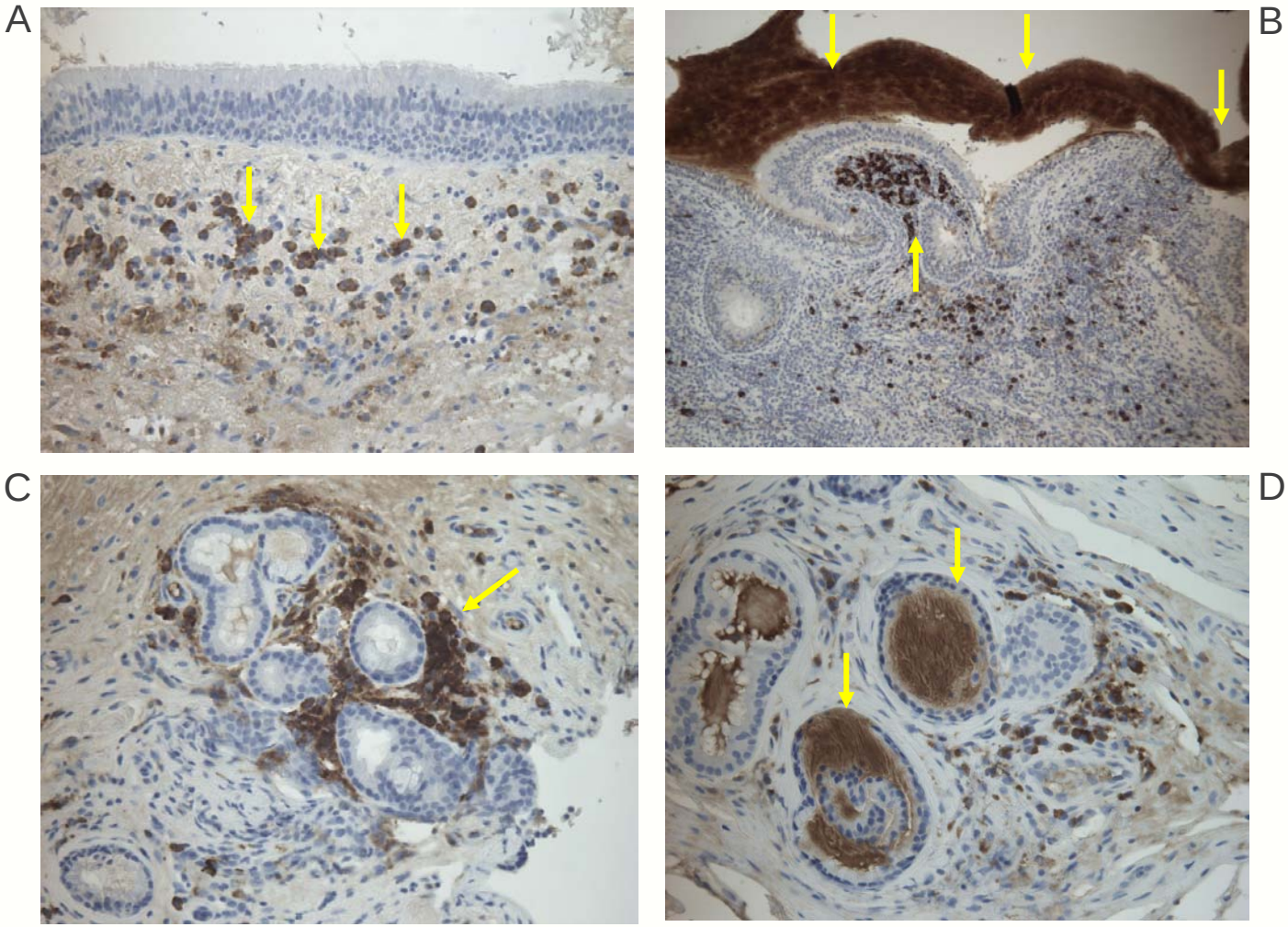
IgA against *P. aeruginosa* alginate in nasal secretions: four infection categories



*p<0.0001 when compared

Aanaes K. et al. JCF 2012

Immunoreaction visualising IgA containing plasma cells in lamina propria and secretion of excretory ducts



Polyclonal antibody to IgA

Aanaes K et al. JCF 2012

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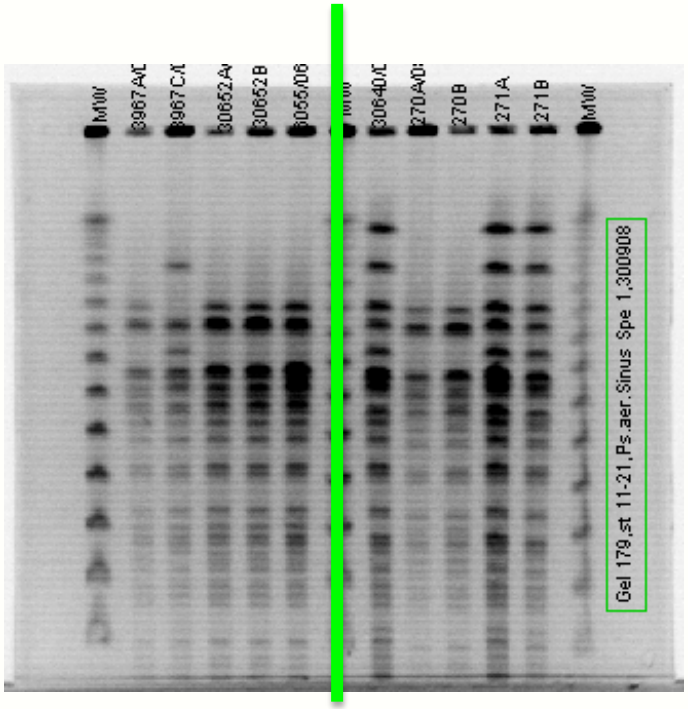
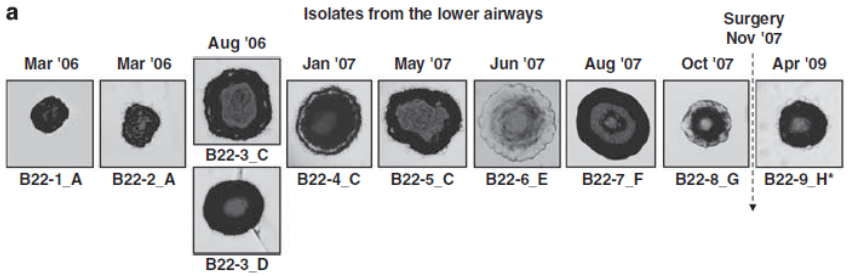
Overlap of genotypes and colony morphotypes in the lungs and the sinuses



PFGE sputum (PA) sinus (PA)



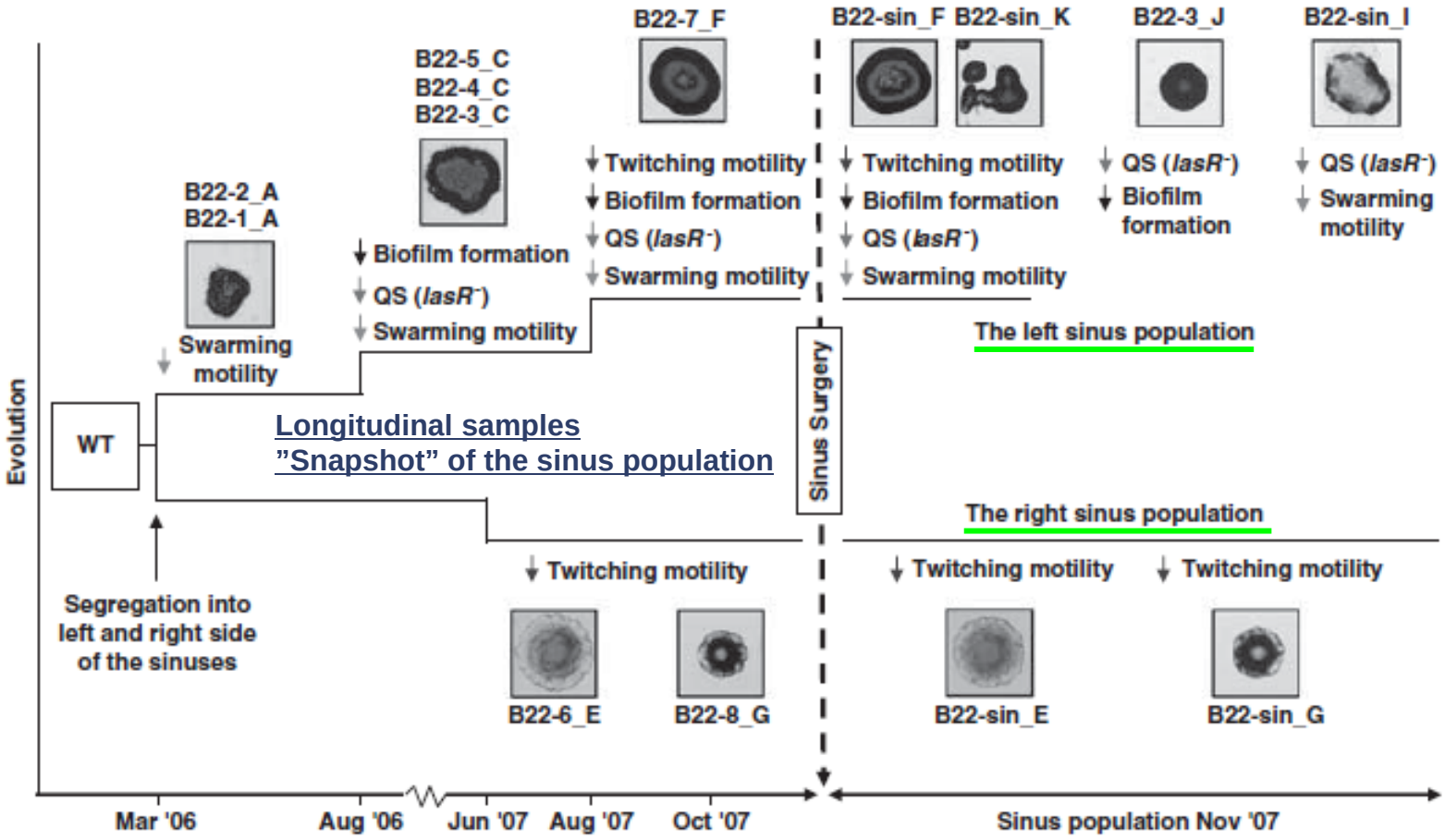
Patient No.	No. of colony morpho-types in the lungs	No. of colony morpho-types in the sinuses	No. of colony morpho-types in both places
B11	6	11	4
B22	7	6	3
B28	4	5	2
B34	4	3	3
B42	5	6	2

Overlap, on an average 3.2 of the *P. aeruginosa* morphotypes found in the lungs were also cultivated from the sinuses

Hansen SK et al.: ISME J. 2012;1:31-45
Johansen HK et al.: JCF 2012

An evolutionary model of the sinus population in a CF child

Evolution of the lung population, two different phenotypic lineages



Child intermittently colonised with *P. aeruginosa*: identity between late lung and sinus isolates

Hansen SK et al.: ISME J. 2012;1:31-45

Conclusions

- Same environment in the sinuses and lungs
- Environmental isolates adapt and evolve in the sinuses, downwards migration of *P. aeruginosa* in the early stages of colonisation/infection
- Different immune responses
    - In the sinuses IgA prevents attraction of PMNs, complement is not activated and the oxidative burst is diminished
    - In the lungs *P. aeruginosa* elicits stimulation of the innate immune system and inflammation leading to high IgG response
- Adaptation
    - Sinuses: aerobic and anaerobic niches
    - Conductive airways: anaerobic niche
    - Respiratory zone: aerobic niche
- The paranasal sinuses is an evolutionary ‘nest’ in early colonisations
- In selected patients FESS and intensive antibiotic therapy can eradicate *P. aeruginosa* from the sinuses, which may prevent or delay transition to chronic lung infection

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- Department of Systems Biology, Danish Technical University, Lyngby
  Søren Molin, Lars Jelsbak, Susse Kirkelund Hansen, Martin Holm Rau, Lei Yang

- Institute for Biomedicine, Panum Institute, University of Copenhagen
  Steen Seier Poulsen

- Danish Technological Institute, Life Science Division, Århus/ Ålborg
  Trine Rolighed Thomsen, Vibeke Rudkjøbing, Tine Yding Wolff

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